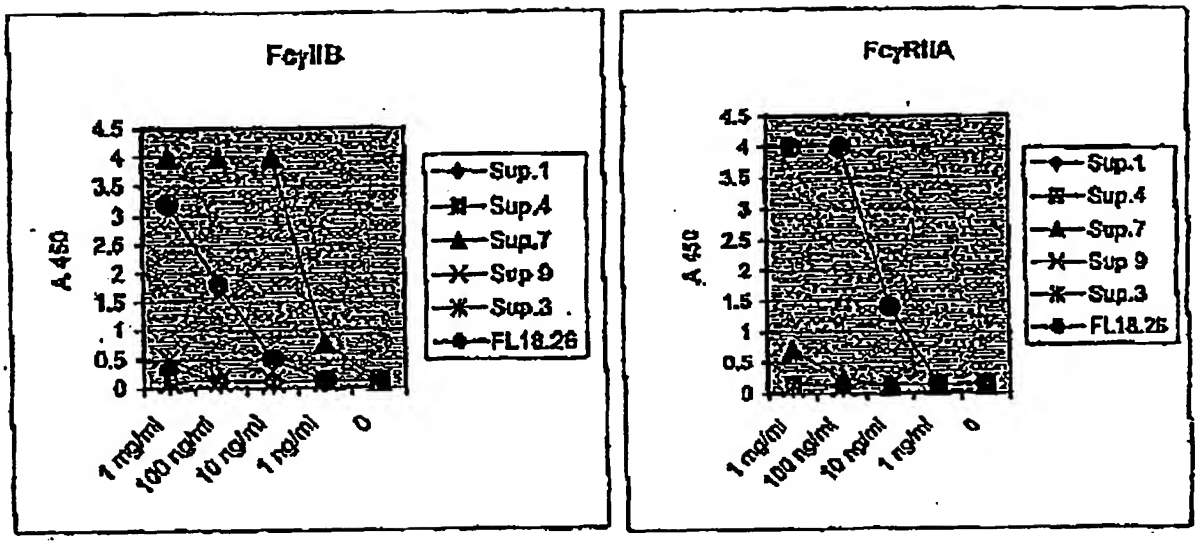


FIG. 1A

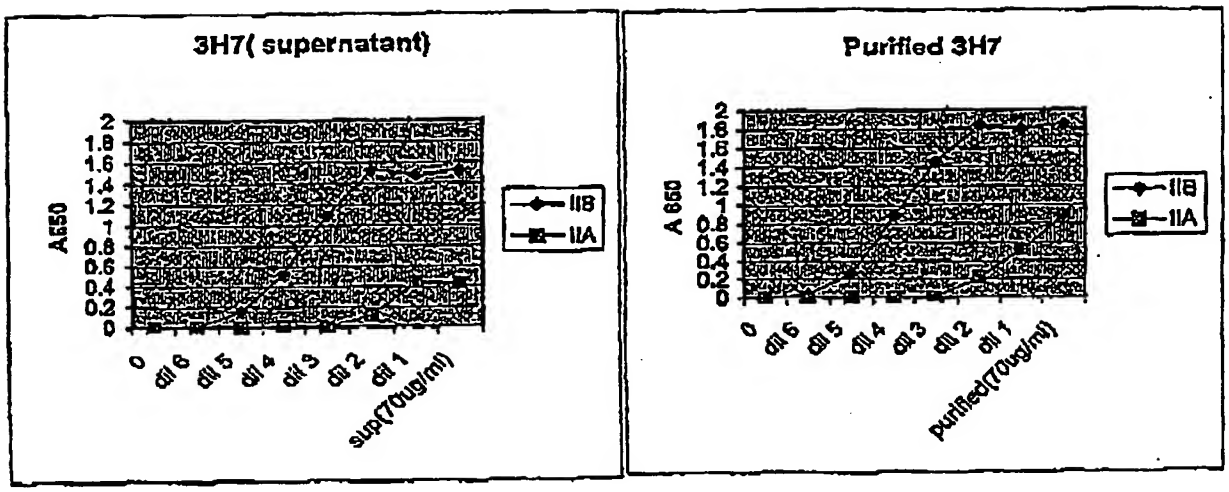

3H7(supernatant)
A650
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IIB
IIA
0
dil 6
dil 5
dil 4
dil 3
dil 2
dil 1
sup(70ug/ml)
Purified 3H7
A 650
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
IIB
IIA
0
dil 6
dil 5
dil 4
dil 3
dil 2
dil 1
purified(70ug/ml)

FIG. 1B

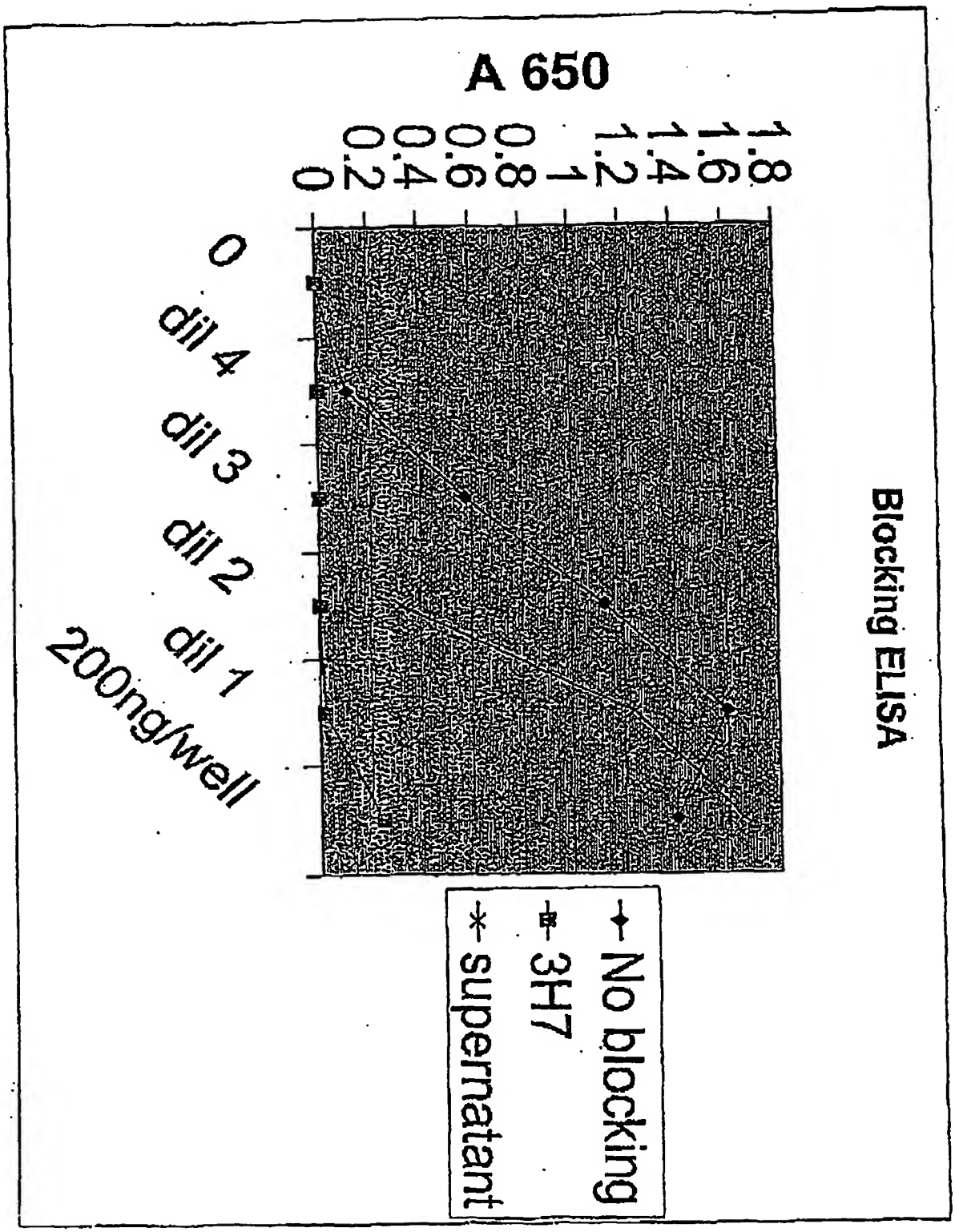

A 650
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
0
dil 4
dil 3
dil 2
dil 1
200ng/well
Blocking ELISA
No blocking
3H7
supernatant

FIG. 2

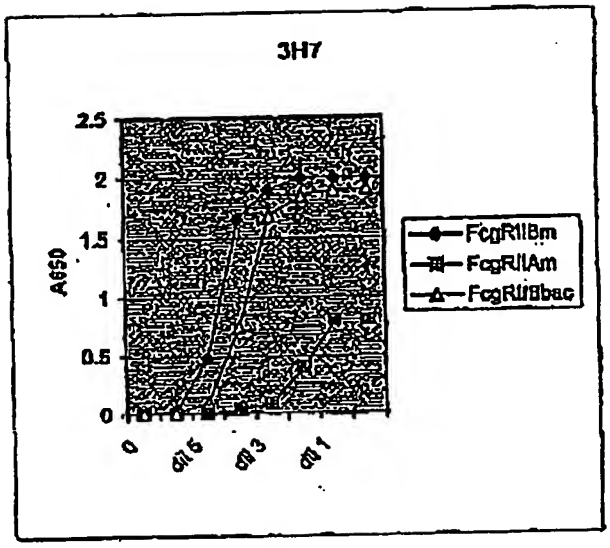


FIG. 3

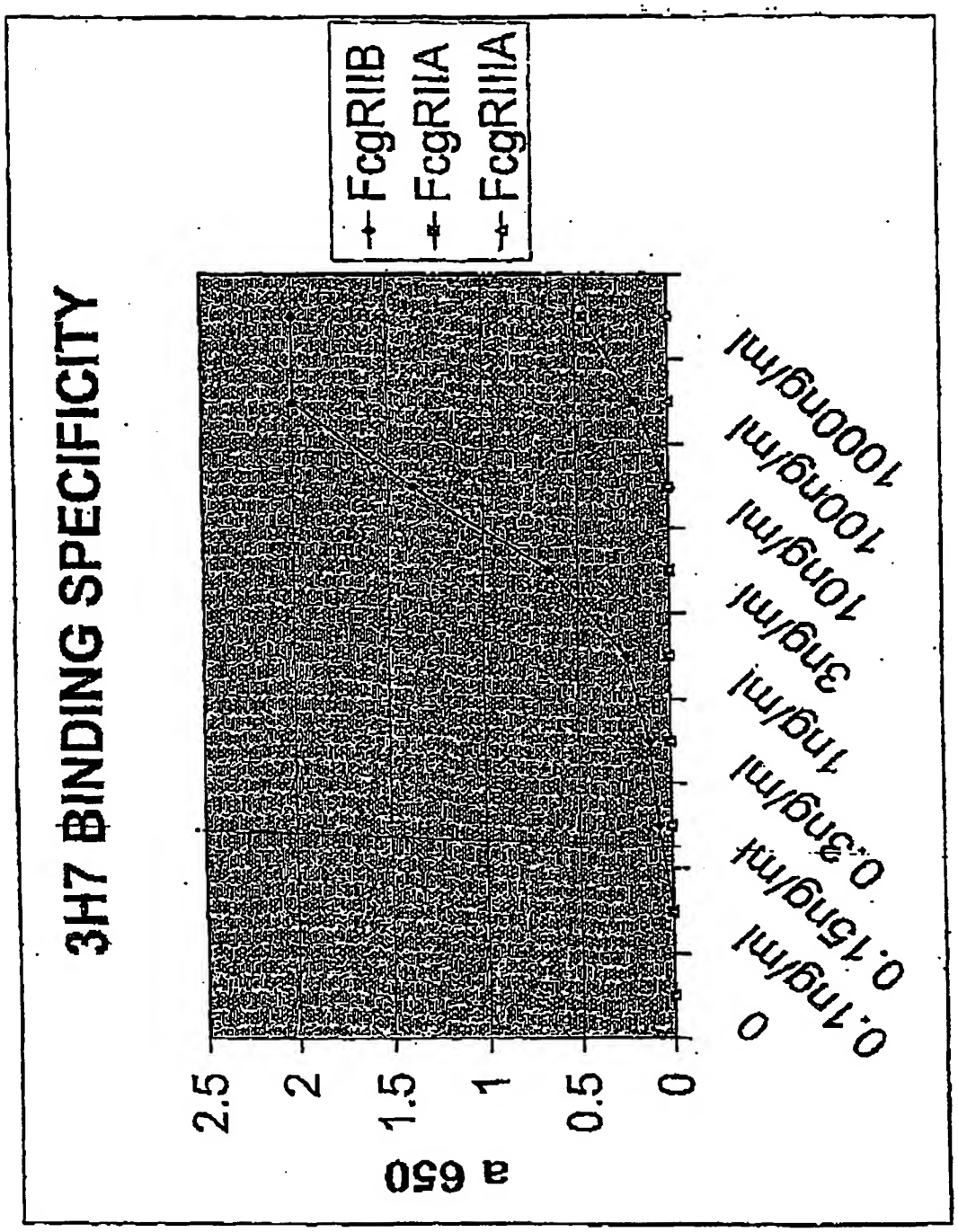
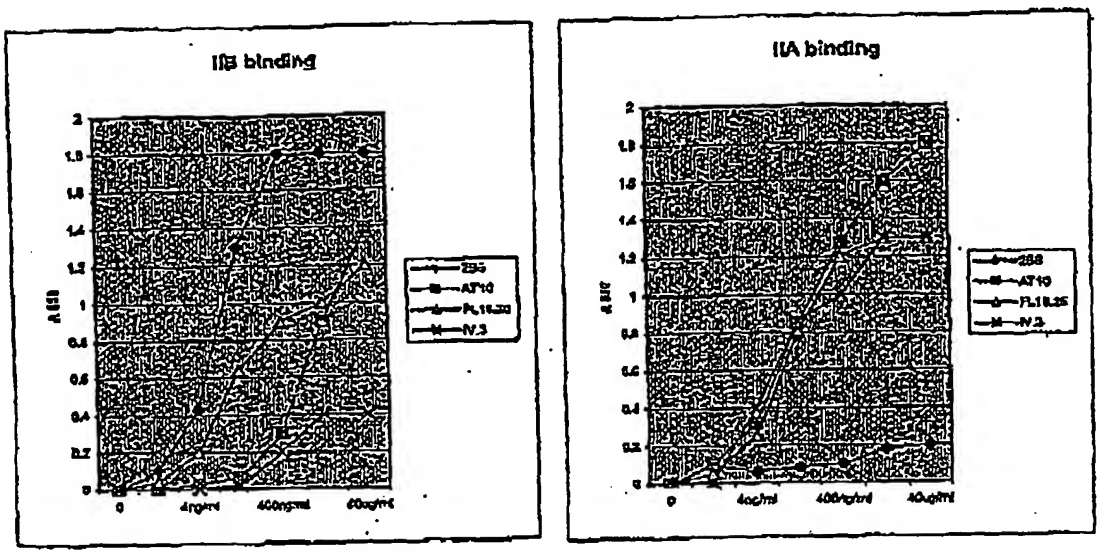

3H7 BINDING SPECIFICITY
FcgRIIB
FcgRIIA
FcgRIIIA
a 650
2.5
2
1.5
1
0.5
0
0
0.1ng/ml
0.15ng/ml
0.3ng/ml
1ng/ml
3ng/ml
10ng/ml
100ng/ml
1000ng/ml

FIG. 4


IIB binding
IIA binding

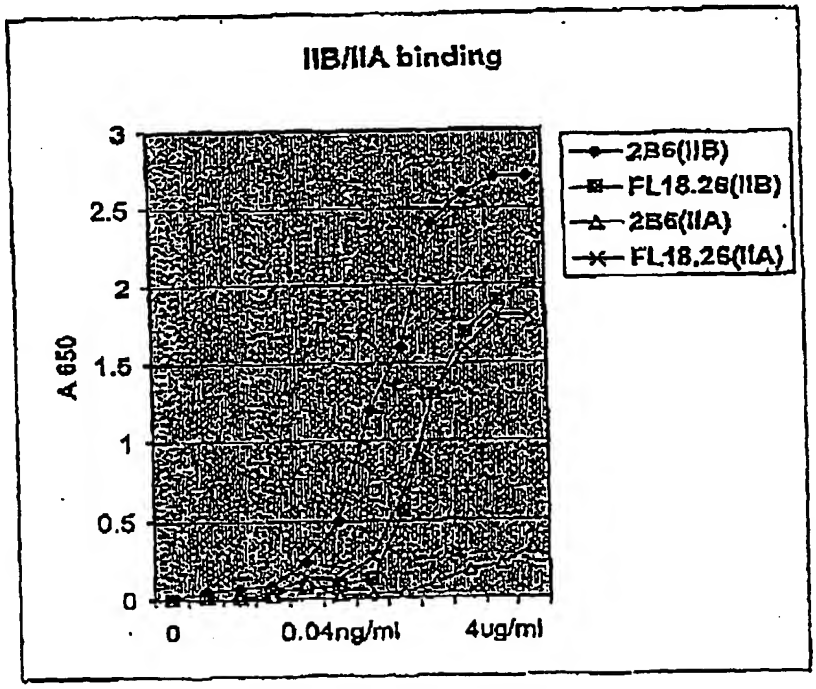
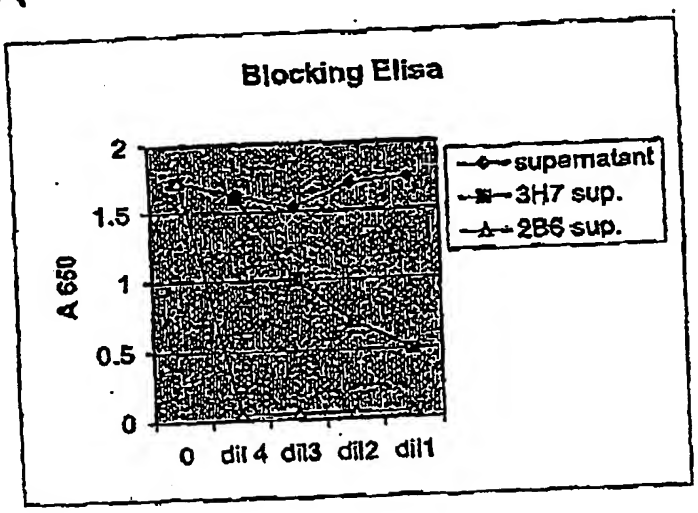

IIB/IIA binding
A 650
3
2.5
2
1.5
1
0.5
0
0
0.04ng/ml
4ug/ml
2B6(IIB)
FL18.26(IIB)
2B6(IIA)
FL18.26(IIA)

FIG. 5

A


Blocking Elisa
A 650
2
1.5
1
0.5
0
0 dil 4 dil3 dil2 dil1
supernatant
3H7 sup.
2B6 sup.

B

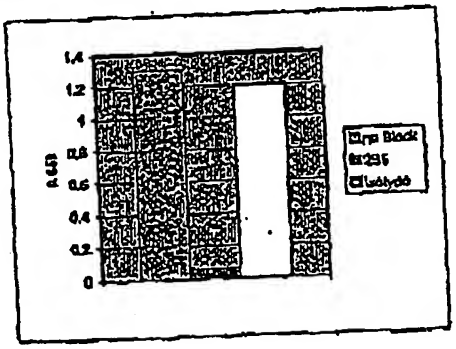
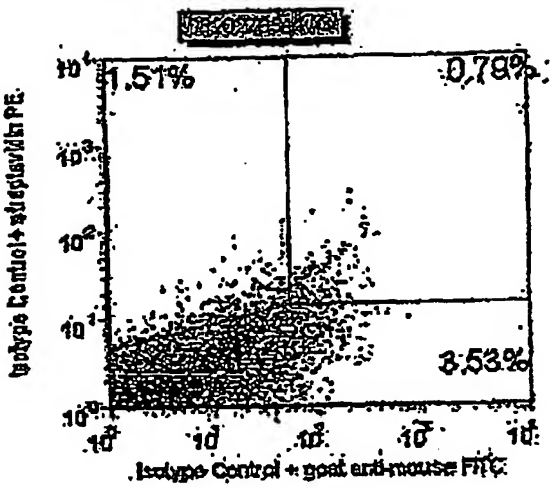
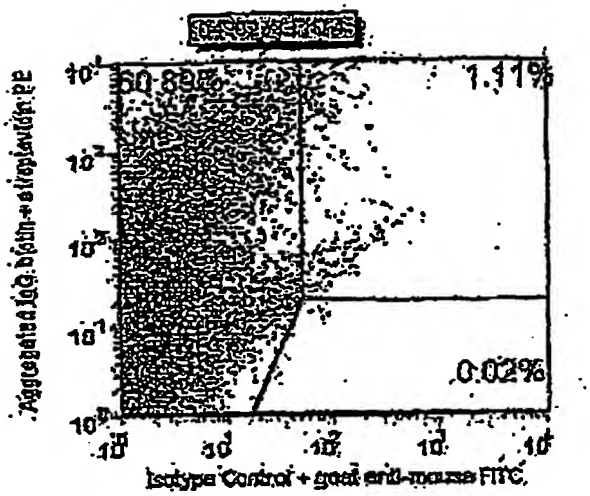

1.4
1.2
1
0.8
0.6
0.4
0.2
0

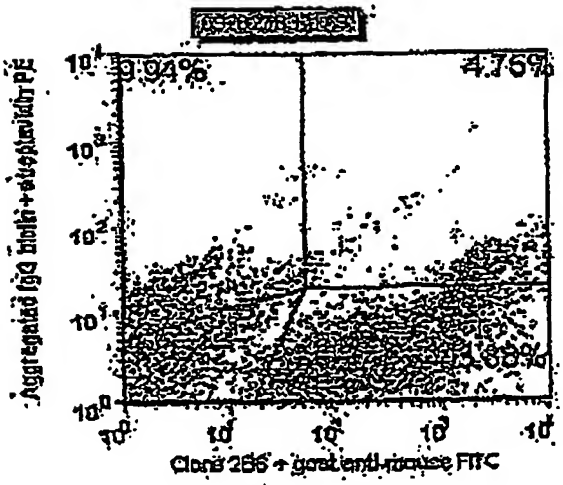
FIG. 6


1.51%
0.79%
3.53%
Isotype Control + goat anti-mouse FITC

A


50.89%
1.11%
0.02%
Isotype Control + goat anti-mouse FITC

B


9.94%
4.76%
Clone 286 + goat anti-mouse FITC

C

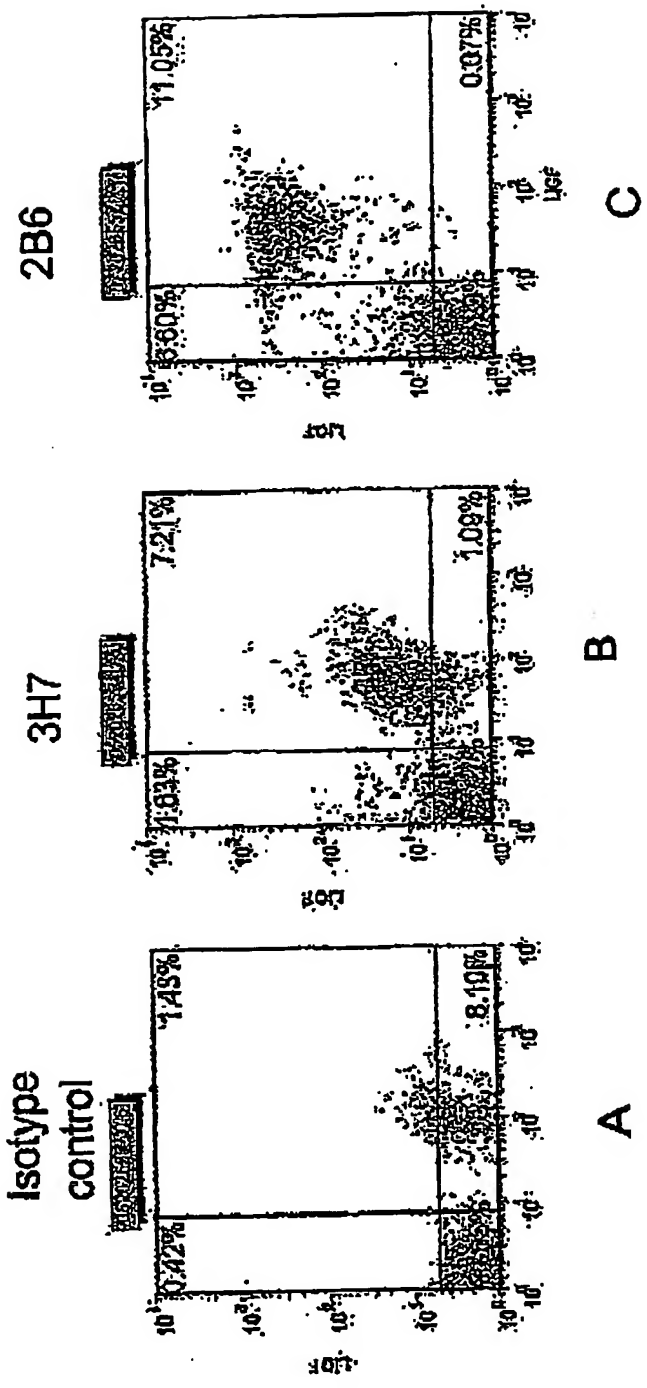

Isotype control
1.43%
0.42%
8.10%
A
3H7
7.21%
1.83%
1.09%
B
2B6
11.05%
0.80%
0.07%
C

FIG. 8

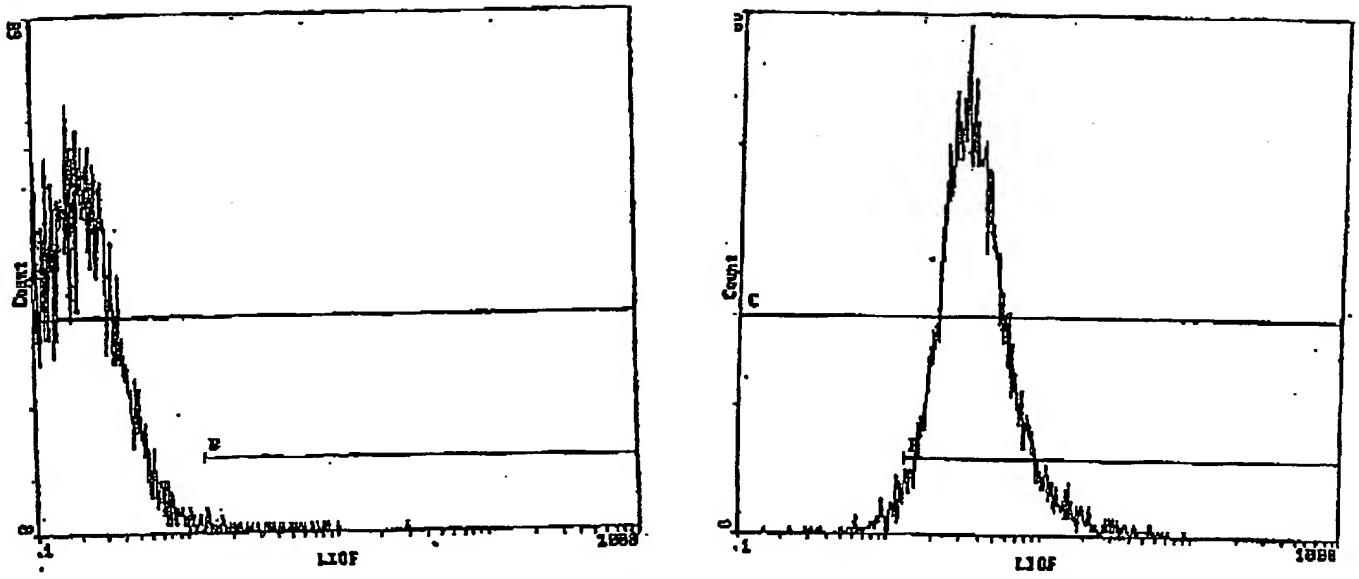


FIG. 9A

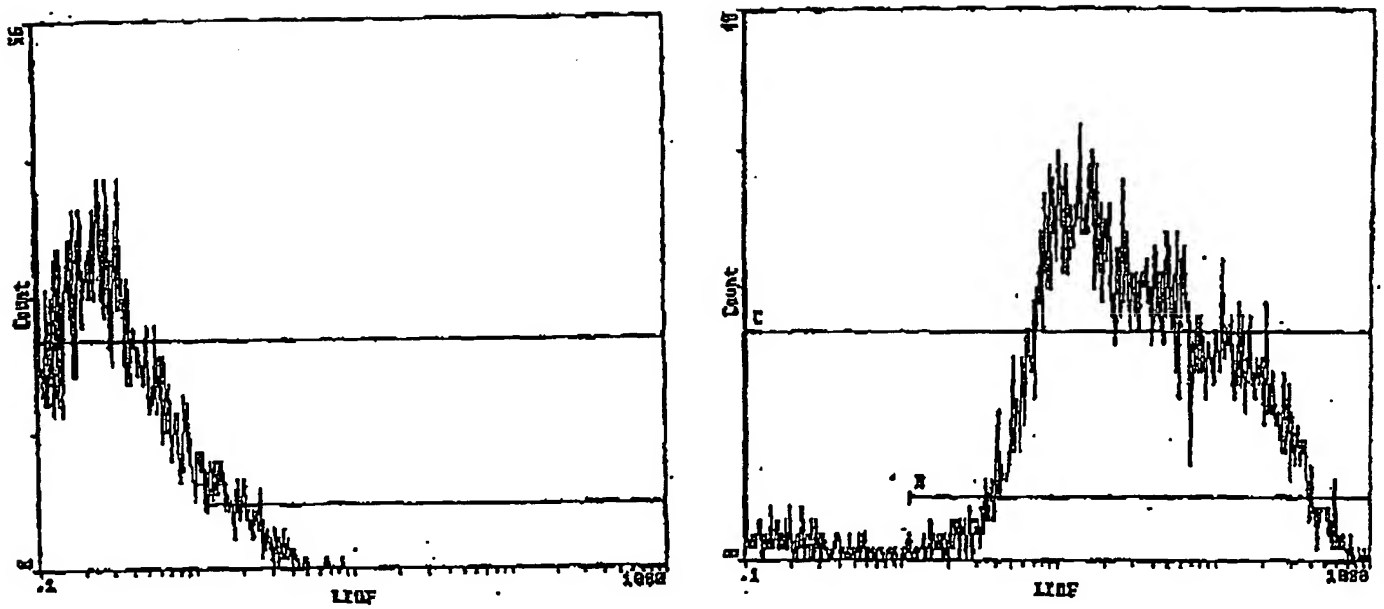


FIG. 9B

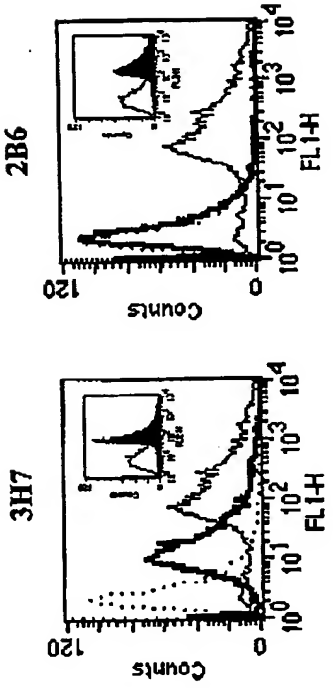


Figure ..... CHO cells expressing huFcγRIIB were incubated with the anti CD32B antibodies, 2B6 or 3H7. Cells were washed and 9 μg/ml of aggregated human IgG were added to the cells on ice. The human aggregated IgG were detected with goat anti human-IgG FITC conjugated. Samples were analyzed by FACS. ..... isotype control + goat anti huIgG-FITC, ——isotype control + aggregated humanIgG + goat anti humanIgG-FITC, ▬ anti-CD32B antibody + aggregated humanIgG + goat anti humanIgG-FITC. The amount of each antibody bound to the receptor on the cells was also detected (inset) on a separate set of samples using a goat anti-mouse PE conjugated antibody.

Fig 11

Figure 11. Human PBMCs were stained with 2B6, 3H7, and IV.3 antibodies, as indicated in the right side of the panel, followed by a goat anti-mouse-Cyanine(Cy5) conjugated antibody (two color staining using anti-CD20-FITC conjugated for B lymphocytes, anti-CD14-PE conjugated for monocytes, anti-CD56-PE conjugated for NK cells and anti-CD16-PE conjugated for granulocytes.

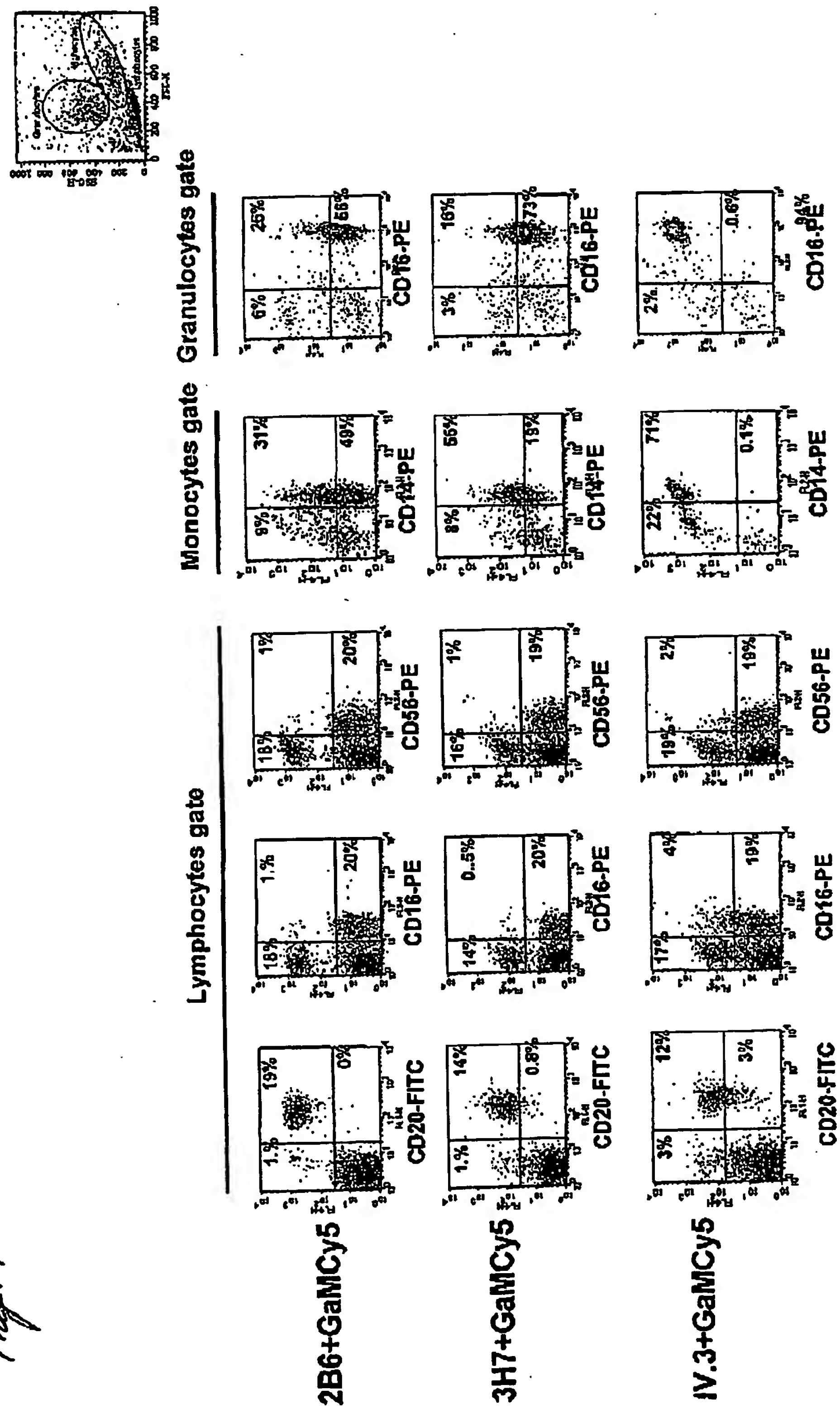
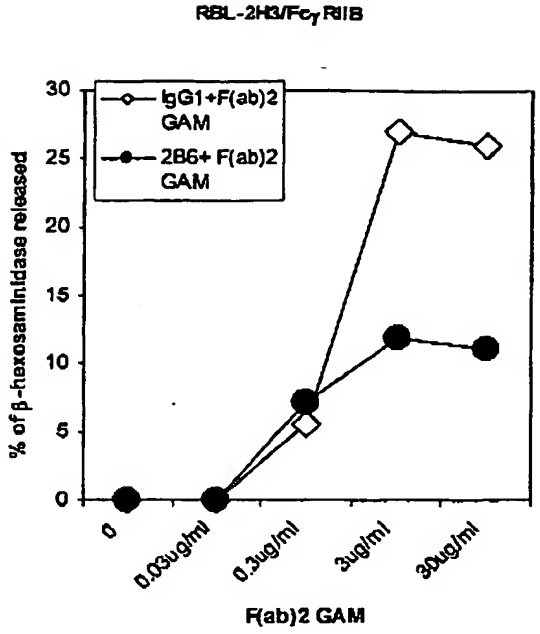


Fig. 11

All also
- legend



12

Figure ..... B-hexosaminidase release induced by goat anti-mouse $F(ab)_2$ fragment (GAM $F(ab)_2$) in RBL-2H3 cells expressing huFcγRIIB. Cells were stimulated with various concentration of GAM $F(ab)_2$ (0.03μg/ml to 30 μg/ml) after sensitization with mouse IgE (0.01 μg/ml) and IgG1 or with purified 2B6 antibody (3 μg/ml) panel. After 1 hour at 37°C the supernatant was collected and the cells were lysed. B-hexosaminidase activity released in the supernatant and within the cells was determined by a colorimetric assay using p-nitrophenyl N-acetyl-β-D-glucosaminide. The released β-hexosaminidase activity was expressed as a percentage of the released activity relative to the total activity.

Expression of Her2neu on the cell surface of ovarian and breast cancer cell lines

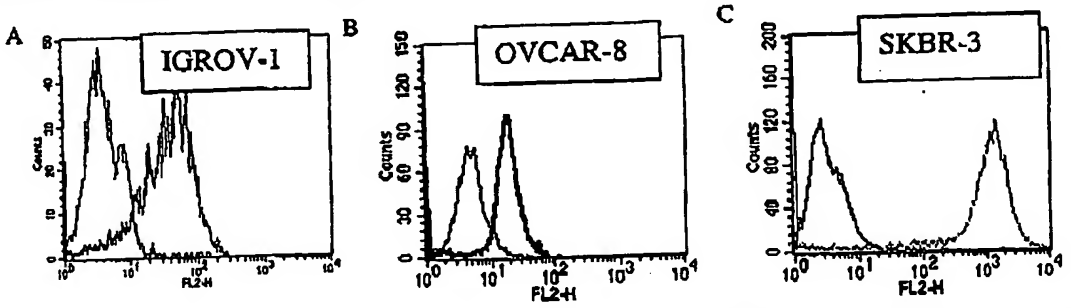


Figure #4: Ovarian and breast carcinoma lines express Her2neu to varying levels. Staining of A) Ovarian IGROV-1 with purified ch4D5, B) Ovarian OVCAR-8 with purified 4D5 antibody, and C). Breast cancer SKBR-3 cells with purified ch4D5 followed by goat anti-human-conjugated to phycocrythrin (PE). The relevant isotype control IgG1 is indicated the left of the staining with anti-Her2neu antibody.

Fig. 14

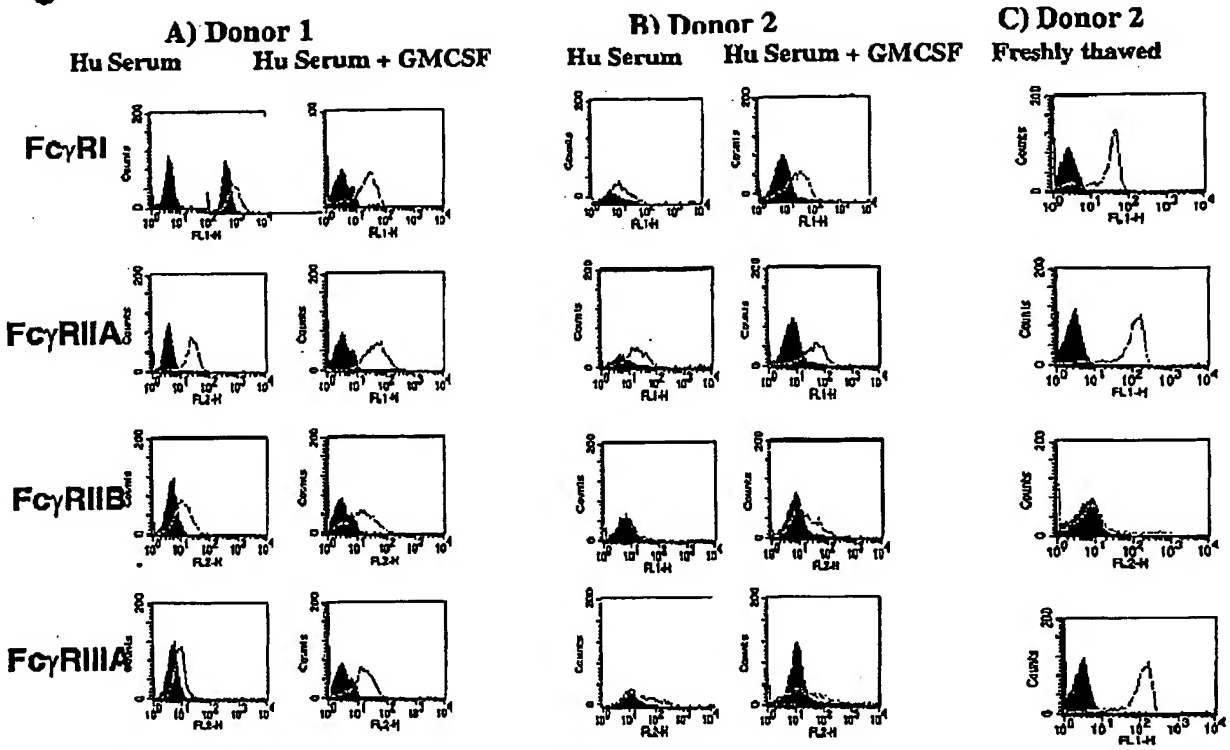


Figure 6: Elutriated monocytes express all FcγRs: A) MDM obtained from donor 1, B) donor 2 were propagated in human serum or human serum and GMCSF and C) Monocytes thawed and stained immediately. Monocyte-derived macrophages were stained with anti-bodies specific for human FcγR receptor, (section C.4). The solid histogram in each plot represents the background staining. The clear histogram within each panel represents the staining with specific anti-human FcγR antibodies.

FIGURE #7

A)

B)

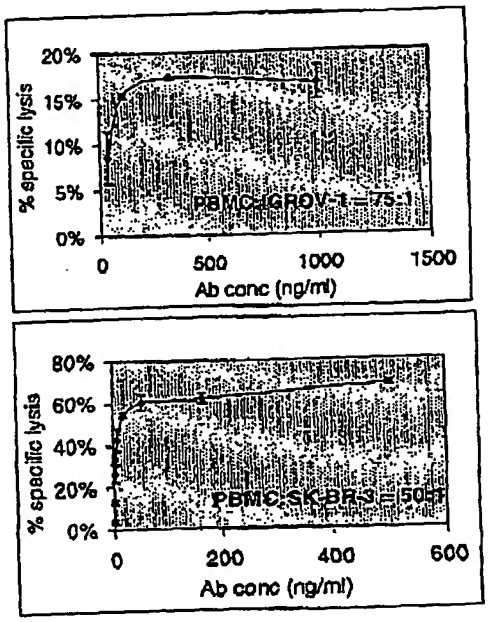


Figure #7: Ch4D5 mediates effective ADCC with ovarian and breast cancer cell lines using PBMC. Specific lysis subtracted from antibody-independent lysis is shown for A) Ovarian tumor cell line, IGROV-1 at an effector: target ratio of 75:1, and for B) Breast tumor cell line SKBR-3 at an effector:target ratio of 50:1 with different concentration of ch4D5 as indicated.

FIGURE #5

Figure #5: Histochemical staining of human ovarian ascites shows tumors cells and other inflammatory cells. A). H & E stain on ascites of a patient with ovarian tumor. Three neoplastic cells can be identified by the irregular size and shape, scattered cytoplasm, and irregular dense nuclei. B). Giemsa stain of unprocessed ascites from a patient with serous tumor of the ovary shows two mesothelial cells placed back to back indicated by short arrows. Also shown is a cluster of five malignant epithelial cells indicated by the long arrow. Erythrocytes are visible in the background. C). Giemsa stain of another patient with serous tumor of the ovary indicating a cluster of cells composed of mesothelial cells, lymphocytes, and epithelial neoplastic cells(arrow).